

PATENT SPECIFICATION

(11) 1 352 420

1352 420

- (21) Application No. 28820/71 (22) Filed 18 June 1971
 (44) Complete Specification published 8 May 1974
 (51) International Classification C07C 129/08; A01N 9/20; A61K 27/00
 (52) Index at acceptance
 C2C 20Y 29X 29Y 30Y 320 342 34Y 366 367
 368 581 62X 790 791 796 79Y KN
 A2D 2L 2M 3A1 3AX 3BX
 (72) Inventors FUMIHIRO YOSHINAGA, RYONOSUKE YOSHIDA,
 TADAOMI SAITO and MASAHIRO TAKEHARA



(54) NOVEL ARGININE DERIVATIVES, THEIR PRODUCTION AND THEIR USE

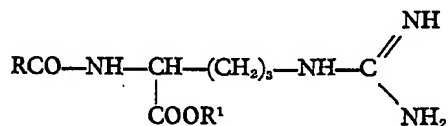
(71) We, AJINOMOTO CO., INC., a corporation organised under the law of Japan, of No. 6, 1-chome, Kyobashi, Chuo-ku, Tokyo, Japan, formerly of No. 7, 1-chome, Takaracho, Chuo-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to novel arginine derivatives, and to combinations of a material other than a detergent and, in association with the material, the arginine derivative as an antimicrobial or germicidal agent.

It is known that materials such as cosmetics, leather goods, rubber goods, paints, foods and animal feeds are easily attacked by microorganisms and hence such materials do not lend themselves to long storage or use.

Various antiseptic or antifungal agents have hitherto been developed and used in the treatment of such materials. However, such previously used agents do not possess both the ability to inhibit completely the growth of microorganisms and the ability not to cause irritation of the skin or not to be toxic.

According to one aspect of the present invention, there is provided a lower alkyl ester of mono-N-higher aliphatic acyl arginine having the formula:



wherein RCO is a higher aliphatic acyl radical containing at least 6 carbon atoms and R¹ is a lower alkyl radical containing up to 4 carbon atoms; or a salt of the ester.

According to another aspect of the present invention, there is provided in combination, a material (other than a detergent) which is susceptible to microorganism attack and, in associa-

tion with the material, a lower alkyl ester of mono-N-higher aliphatic acyl arginine, or a salt of the ester.

It has been found that the arginine derivatives of the present invention possess adequate water solubility, adequate surface activity and good antiseptic, medicinal, preservative, bactericidal, bacteriostatic, germicidal or fungicidal properties. However, it has been found that such agents do not cause any significant skin irritation and skin troubles.

The arginine derivatives can be prepared, in accordance with a further aspect of the present invention, by a process which comprises reacting arginine with a higher aliphatic acyl halide of formula RCOX where RCO is as defined above and X is a halogen atom, in an alkaline aqueous solution; and esterifying the resulting mono-N-higher aliphatic acyl arginine so as to introduce the lower alkyl radical R¹ as defined above; and, if desired, converting the resulting ester to a salt of the ester.

A yet further aspect of the present invention provides a method of treating a material other than a detergent to reduce or prevent microorganism attack, which comprises applying to, or incorporating in, the material an arginine derivative according to the first-mentioned aspect of the present invention.

Either the optically active L- or D-form or the racemic form of the arginine component is effective. However, the optically active L-form is preferred.

Examples of salts of the lower alkyl esters of N-higher aliphatic acyl arginine include salts with a mineral acid such as hydrochloric acid or sulphuric acid, and salts with an organic acid such as an optically active or inactive α-pyrrolidone-carboxylic acid, an optically active or inactive acidic amino acid (e.g. glutamic acid and aspartic acid), lactic acid, citric acid and acetic acid. The use of a hydrochloride salt or of the salt with DL- or L-α-pyrrolidone-carboxylic acid is particularly convenient in view of their crystalline nature.

Preferably the higher aliphatic acyl radical

is a saturated or unsaturated fatty acid radical containing from 6 to 20 carbon atoms.

More preferably the higher aliphatic acyl radical is a lauroyl, cocoyl or stearoyl radical.

- 5 Preferably the lower alkyl radical R¹ is a methyl, ethyl, propyl, or butyl radical.

Representative examples of lower alkyl esters of mono-N-higher aliphatic acyl arginine and salts thereof include the following: N^α-cocoyl-L-arginine ethyl ester pyrrolidone-carboxylate; N^α-cocoyl-L-arginine methyl ester pyrrolidone-carboxylate; N^α-lauroyl-L-arginine methyl ester hydrochloride; and N^α-stearoyl-L-arginine methyl ester hydrochloride.

- 10 The abbreviation used in this specification is as follows: "cocoyl" for coconut oil fatty acid residue. The active agents which may be employed in the present invention are not limited to these examples.

- 20 The lower alkyl esters of mono-N-higher

aliphatic arginine (and their salts) show a good inhibitory effect against microorganisms which possess relatively strong resistance not only to gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, but also to gram negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris*. The bactericidal or inhibitory effect of some of the compounds of the present invention, in comparison with that of "Hyamine" (a product of Rohm & Haas Co., Inc.) and hexachlorophene, is illustrated in the following Table 1. The word "Hyamine" is a registered Trade Mark. The phenol coefficient of N^α-cocoyl-L-arginine ethyl ester pyrrolidonecarboxylate is high; for example, this phenol coefficient toward *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* is 170, 85 and 250 respectively.

TABLE 1

Agents		Microorganism No.						
		1	2	3	4	5	6	7
N ^α -cocoyl-L-arginine ethyl ester pyrrolidone carboxylate		75~100	150~200	50~100	50~100	400~500	100~150	2000~2300
N ^α -cocoyl-L-arginine methyl ester pyrrolidone carboxylate		75~100	150~200	50~100	50~100	400~500	100~150	2000~2300
Reference Agents	"Hyamine" (benzethonium chloride)	50~100	150~200	50~100	25~100	400~500	100~150	800~1000
	"G-11" (hexachlorophene)	1200~1500	1200~1500	1000~1200	250~300	400~500	100~150	800~1000

Note: The numerical values in Table 1 indicate the inhibiting concentration of the agent against the growth of microorganism in γ/cc., by contacting each strain with the aqueous solution of ten minutes.

The microorganisms, culture media and preparation of cultured cells employed in the test, and the method of calculating bactericidal activity are as follows:

- 45 (a) Microorganisms employed:—
 1. *Escherichia coli* (ATCC 3655)
 2. *Pseudomonas aeruginosa* (IAM 1002)
 3. *Proteus vulgaris* (IAM 1025)
 50 4. *Staphylococcus aureus* (ATCC 6538P)
 5. *Bacillus subtilis* (ATCC 6633)
 6. *Candida albicans* (AJ 14146)
 7. *Aspergillus niger* (ATCC 9642).
 The term "IAM" is an abbreviation of the

Institute of Applied Microbiology, Tokyo University, Japan; and the term "AJ" is an abbreviation of Ajinomoto Co., Inc. The AJ-number is that accorded the microorganism concerned by our corporation from whom the microorganism is freely available upon request.

- (b) Culture media employed:—
 1. Meat extract 1.0%, polypeptone 1.0%, NaCl 0.25%, pH 7.0 (used for strains 1—5).
 2. Yeast extract 0.3%, meat extract 0.3%, polypeptone 0.5%, glucose 1.0%, pH 6.2 (used for strains 6 and 7).

(c) Preparation of cultured cells:—

5 Strains 1 to 6 were cultured statically at 31° C for 20 to 24 hours in test tubes into which the above-mentioned media had been introduced, whereas strain 7 was cultured at 31° C for 4 days on a yeast-malt agar slant.

(d) Assay method of bactericidal activity:—

10 0.5 ml. of each of the above-mentioned cultured cells was introduced into different test tubes each containing 10 ml. of sterilized aqueous solution containing a particular concentration of the various agents. After the cells had been contacted with the agent, one loopful of cell suspension was spread, in the case of strains 1 to 5, on a nutrient-bouillon agar plate, and, in the case of strains 6 and 7 on a yeast-malt agar plate. After cultivation at 31° C

for 48 hours, the cells were examined to see whether they were alive or dead.

The mono-N-higher aliphatic acyl arginine derivatives of the present invention possess a powerful anti-bacterial activity against microorganisms possessing relatively strong resistance to various known antimicrobial compounds, examples of such microorganisms being *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. For example, the bacteriostatic or inhibitory effect of N^a-lauroyl-L-arginine methyl ester pyrrolidone carboxylate in comparison with that of 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide, palmityl-L-lysyl-L-lysine methyl ester dihydrochloride, streptomycin, penicillin, sorbic acid and lauroyl sarcosine is illustrated in the following Table 2.

TABLE 2

Microorganism	1	2	3	4	5	6
Agent						
1. N ^a -lauroyl-L-arginine methyl ester pyrrolidone carboxylate	10~ 100	10~ 100	10~ 100	100~ 1000	10~ 100	10~ 100
2. 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (referred to as "AF-2")	10~ 100	10~ 100	10~ 100	1000<	1000<	1000<
3. Palmitoyl-L-lysyl-L-lysine methyl ester dihydrochloride	10~ 100	<10	<10	100~ 1000	1000<	1000<
4. Streptomycin	<10	10~ 100	—	1000<	1000<	1000<
5. Lauroyl sarcosine	1000	100	100~ 1000	1000<	1000<	1000<
6. Penicillin	10~					
7. Sorbic acid	1000<	1000<	—	1000<	1000<	1000

The figures in Table 2 represent the concentration (γ/ml) of respective agents which causes the growth inhibition of the respective

microorganism.

Microorganisms tested, culture conditions and culture medium are as follows:

20

25

30

40

No.	Microorganism	Culture conditions	Time for preculti- vation	Medium
1	<i>Escherichia coli</i> (ATCC 3655)	37°C, 3 days	24 hr	Nutrient - bouillon pH 7
2	<i>Staphylococcus aureus</i> (ATCC 6538P)	37°C, 3 days	24 hr	
3	<i>Bacillus subtilis</i> (ATCC 6633)	37°C, 3 days	24 hr	
4	<i>Pseudomonas aeruginosa</i> (IAM 1002)	30°C, 3 days	24 hr	
5	<i>Candida albicans</i> (AJ 14146)	25°C, 3 days	24 hr	Koji added glucose and yeast pH 5.8
6	<i>Aspergillus niger</i> (ATCC 9642)	25°C, 3 days	48 hr	

The agents of the present invention either possess only a small toxicity or are non-toxic; consequently they do not cause any significant skin irritation. For example, in an acute oral toxicity test (LD₅₀) carried out on mice, the LD₅₀ value of N^c-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate is 10.75 g/kg body weight. Accordingly, such an agent is not harmful to man or beast under normal use. In a further test, this amino acid derivative was well mixed with polyethylene glycol and then spread on the gauze region of a sticking plaster, and the plaster then applied to human skin for 24 hours. Almost no irritation of the skin was observed. Moreover, in view of the fact that the agents are water-soluble cationic surface active agents, they have a significant detergent effect on account of their strong foaming action.

The lower alkyl esters of mono-N-higher aliphatic acyl arginine, or salts thereof, can be used as a liquid, a paste, a powder or a solid, for the purpose of, for example, disinfecting and sterilizing food, in the industrial field and in the farming and gardening field. The arginine esters can also be incorporated in cosmetic preparations, or used as detergents for vegetables and fruit, or used as disinfectant agents for animals.

Moreover, as the arginine esters have a strong emulsifying power toward cosmetics, are water-soluble, and their solubility in an aqueous phase is higher than that in an oil phase, they show an appreciable antiseptic effect even when added in a small amount to cosmetics. Furthermore, they are capable of strongly penetrating fabrics. Accordingly, the application of the agents is wide.

Thus, for example, the arginine esters or

their salts can be compounded with, applied to or sprayed on, the material to be protected which can be food, cosmetics, fibrous goods, leather goods or paint. The arginine ester is usually originally in the form of a liquid, powder or emulsion.

Another use to which the arginine esters and their salts can be put is as an active ingredient in an antiseptic for preventing "hiochi putrefaction" during brewing or similar operations.

The phenomenon of "hiochi putrefaction" makes "sake" (Japanese wine), synthetic sake which contains partially brewed alcohol, or the products of brewed alcohol such as "mirin" (a sweet kind of "sake"), impossible to drink because of a white muddiness and rancidity which occurs during storage or after bottling.

Generally, "hiochi putrefaction" is caused by *Lactobacillus heterohiochi*, *Lactobacillus janonicus* or *Lactobacillus homohiochi* which comes from brewing.

Examples 6 and 7 of the following Examples indicate the antibacterial activity of N^c-lauroyl-L-arginine-methyl ester pyrrolidone carboxylate in comparison with that of salicylic acid which has conventionally been used for preventing "hiochi putrefaction".

Moreover, it is confirmed that the arginine derivatives of the present invention adhered to a mucose in an oral cavity and were disinfectant in it for a considerable time. It was found that the arginine derivatives have marked antibacterial activity against both a bacterium belonging to the genus *Lactobacillus*, a main pathogen of dental caries, and a bacterium belonging to genus *Staphylococcus*, a main pathogen of alveolar pyorrhea.

The following Table 3 shows the results of antibacterial tests involving certain arginine derivatives against *Lactobacillus fermenti*-36 ATCC 9338, and *Streptococcus faecalis*

ATCC 8083, and *Staphylococcus aureus* ATCC 65389.

Table 3 also shows the antibacterial activity of sodium N-lauroyl sarcosinate as a control.

TABLE 3

Agent	(*) <i>Lactobacillus</i> <i>fermenti</i>	<i>Streptococcus</i> <i>faecalis</i>	<i>Staphyl-</i> <i>aureus</i>
N ^α -cocoyl-L-arginine methyl ester hydrochloride	14	36	5
N ^α -cocoyl-L-arginine methyl ester PCA (**)	18	38	12
N ^α -lauroyl-L-arginine ethyl ester PCA (**)	21	45	8
Sodium-N-lauroyl sarcosinate (control)	70	49	80

The numerical values in Table 3 represent the concentration (γ/ml) of agent which causes a reduction of 50% of the bacterial growth.

(*) The growth of *Lactobacillus* and *Streptococcus* was measured after 24 hours with a standing culture at 37°C, and that of *Staphylococcus* was measured after 48 hours at 31°C.

(**) DL-Pyrrolidone carboxylic acid = PCA.

- 10 It is evident from Table 3 that one of the three agents according to the present invention is more effective than the control.

- 15 The three agents possess nearly the same foaming power, which is a desirable surface active action, when the agent is incorporated in a dentifrice, as shown in the following

Table 4 which includes, as the control, sodium-N-lauroyl sarcosinate. The foaming powers of 0 minute value are determined by the method of JISK 3362. From the results, it can be seen that each of the specified agents possesses satisfactory properties for use as a component in a dentifrice.

20

TABLE 4

Agent	Foaming power (mm)
N ^α -cocoyl-L-arginine methyl ester PCA salt	190
N ^α -cocoyl-L-arginine ethyl ester PCA salt	192
N ^α -cocoyl-L-arginine methyl ester hydrochloride	185
Sodium-N-lauroyl sarcosinate (control)	200

In the following Examples, references to parts are to parts by weight.

Example 1.

This Example relates to an examination of the effect of a 0.1% solution of N^c-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate (CAE—P) on the disinfection and washing of peoples' fingers.

Various types and quantities of bacteria were found to exist on the fingers of fifteen persons selected as a panel.

Each member of the panel immersed his hands and fore-arms, up to a point 35 cm

from the tip of the middle finger, in a wash-bowl containing 2 l. of tap water, and then repeated the same thing for a minute in 0.1% N^c-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate solution. Finally each member washed his hands and forearms in 2 l. of sterilized water.

The number of living cells in these washings was counted after the performance of all members. Separately, another group of fifteen members carried out the same experiment as control except that they used tap water instead of 0.1% N^c-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate solution.

	Number of living cells (in 0.1 ml)	
	Before washing	After washing
With 0.1% CAE-P	341	16
Without 0.1% CAE-P	348	289

Example 2.

A bath preparation was prepared by mixing the following components in the weights indicated:—

N ^c -cocoyl-L-arginine ethyl ester-DL- α -pyrrolidone carboxylate	500 g.
Sodium iodide	1.0
Sodium bromide	0.6
Lithium carbonate	0.6
Manganese sulphate	0.01
Iron sulphate	0.01
Potassium chloride	15.0
Calcium chloride	40.0
Magnesium sulphate	66.4
Magnesium chloride	96.0
Sodium chloride	280.5

Example 3.

A preventive cleansing agent was prepared by mixing the following components in the weights shown:—

N ^c -lauroyl-L-arginine ethyl ester-DL- α -pyrrolidone carboxylate	3.0% (by weight)
Triethanolamine	2.0% " "
Perfume	0.3% " "
Water	94.7% " "

Example 4.

A toilet water was prepared as follows.

Ten parts of ethanol, 0.05 part of gum tragacanth, 5 parts of propylene glycol and 1 part of N^c-lauroyl-L-arginine methyl ester-DL- α -pyrrolidone carboxylate were mixed with 85 parts of water.

Separately a control toilet water containing polyoxyethylene sorbitan monolaurate instead of the arginine derivative was prepared in the same way.

In the toilet water containing the arginine derivative no change in quality was found on storage for one month in a room at 30° C and at a relative humidity of 90%.

The control toilet water showed the growth of mould.

Example 5.

0.5 Ml of an aqueous 3% solution of N^c-lauroyl-L-arginine ethyl ester DL- α -pyrrolidone carboxylate was sprayed per 100 cm² of surface of dressed oxhide.

In the sprayed dressed oxhide no change in quality was found on storage for a month at 30° C and at a relative humidity of 90%.

A control dressed oxhide, not treated with arginine derivative, showed the growth of mould.

Example 6.

An aqueous culture medium comprising 100 ml of "sake" (Japanese wine) (16% alcohol concentration) and 0.8 g. of beef liver extract was adjusted to pH 5.0.

After sterilization, N^c-lauroyl-L-arginine methyl ester pyrrolidone carboxylate was added to different aliquots of the medium in concentrations of 1 γ /ml, 10 γ /ml, or 50 γ /ml. Three strains of hiochi bacteria listed in Table 5 were introduced to the media and cultured at 30° C. Table 5 indicates the visible growth after 3 weeks culture in the case of the 10 and 50 γ /ml concentrations. Salicylic acid (LD₅₀ orally in rat: 0.9 g/kg) was used as a control (instead of the arginine derivative) in the Example.

TABLE 5

hiochi bacteria	<i>Lactobacillus-heterohiochii</i>		<i>Lactobacillus-japonicus</i>		<i>Lactobacillus-homohiochii</i>	
concentration	10γ	50γ	10γ	50γ	10γ	50γ
N-lauroyl-L-arginine methyl ester pyrrolidone carboxylate	—	—	+	—	+	—
Salicylic acid	+	+	+	+	+	+

— : Cell growth not detected.
+ : Cell growth clearly observed.

Example 7.

- 5 To "Sake" A (17% alcohol content) was added 0.001% of N^c-cocoyl-L-arginine ethyl ester pyrrolidone carboxylate and to "Sake" B (17% alcohol content) was added 0.001% of Na salicylate. The two drinks were stored at 30° C ± 2° C, and then the flavour and taste were compared to each other.
- 10 "Sake" A did not turn at all sour even after storage for six months, whereas "Sake" B began to turn sour approximately after a month.

Example 8.

- 15 A tooth paste was prepared by mixing the following components in the quantities shown:—

	Dicalcium phosphate 2H ₂ O	44.5% (by weight)
20	Gum tragacanth	2.0% " "
	Glycerol	18.5% " "
	N ^c -cocoyl-L-arginine ethyl ester-DL-α-pyrrolidone carboxylate	3.0% " "
25	Saccharin	0.4% " "
	Flavour material	1.0% " "
	Water	30.6% " "

Example 9.

- 30 A wet dentifrice was prepared by mixing the following components in the quantities shown:—

	Calcium carbonate precipitate	70.2% (by weight)
35	Sodium carboxymethyl cellulose	22.0% " "
	N ^c -lauroyl-L-arginine methyl ester DL-α-pyrrolidone carboxylate	3.0% " "
40		

Saccharin	0.5%	" "
Flavouring material	1.3%	" "
Water	3.0%	" "

Example 10.

Preparation of N^c-cocoyl-arginine ethyl ester DL-α-pyrrolidone carboxylate.

35.0 G. (0.2 mole) of L-arginine were dissolved in 200 ml of acetone and 150 ml of water, and then the resulting solution added dropwise, while cooled at 10—20° C, stirring and adjusting to pH 11.5—12.0 with 8N sodium hydroxide, to 40 g. (0.18 mole) of cocoyl chloride (coconut oil fatty acid chloride). The reaction mixture was neutralized with 6N HCl to pH 5.0 and it was then added to 300 ml of cold water.

The precipitate which separated out was filtered and dried: 50 g. of crude crystalline N^c-cocoyl-L-arginine was obtained.

Yield: 77.9%, m.p. 230—235° C.

35.6 G. (0.1 mole) of the N^c-cocoyl-L-arginine was saturated with 200 ml of ethanol solution containing hydrogen chloride and allowed to stand overnight at room temperature. The insoluble material of the resulting reaction mixture was filtered off. The filtrate was concentrated under reduced pressure. The residue was dissolved in 200 ml of ethyl acetate and then added to triethylamine under cooling. The organic solvent layer was washed with water and then added to 12.9 g. of DL-α-pyrrolidone carboxylic acid under heating at 30° C. Thereafter, the reaction mixture was filtered to remove a small amount of insoluble material. The filtrate was concentrated under reduced pressure, and the residue was recrystallized from ethanol. 12.4 G. of white crystals of N^c-cocoyl-L-arginine ethyl ester DL-α-pyrrolidone carboxylate were obtained; the crystals had a m.p. of 181—184° C (dec.).

Our copending British Patent Application No. 60960/70 (Serial No. 1,290,067) claims, *inter alia*, an antimicrobial detergent composi-

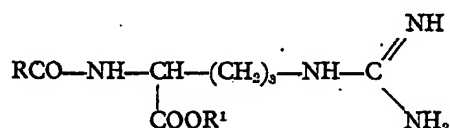
tion comprising a detergent and, in association therewith as an antimicrobial agent, an alkyl ester of mono-N-higher aliphatic acyl arginine, or a salt thereof, where the alkyl group contains up to 4 carbon atoms and the higher aliphatic acyl group is a saturated or unsaturated fatty acid radical containing from 6 to 20 carbon atoms; and we make no claim to such agents when in association with a detergent.

We are aware of The Preservatives in Food Regulations 1971 Statutory Instrument No. 882 and we make no claim to use the invention in contravention of the law.

Furthermore, we make no claim to the use of the invention in a method for the treatment or prevention of disease in human beings involving the treatment thereof.

Subject to the foregoing disclaimers, WHAT WE CLAIM IS:—

1. A lower alkyl ester of mono-N-higher aliphatic acyl arginine having the formula:



wherein RCO is a higher aliphatic acyl radical containing at least 6 carbon atoms, and R¹ is a lower alkyl radical containing up to 4 carbon atoms; or a salt of the ester.

2. A compound as claimed in Claim 1, wherein the higher aliphatic acyl radical is a saturated or unsaturated fatty acid radical containing from 6 to 20 carbon atoms.

3. A compound as claimed in Claim 2, wherein the higher aliphatic acyl radical is a lauroyl, cocoyl or stearyl radical.

4. A compound as claimed in Claim 1, 2 or 3, wherein the lower alkyl radical R¹ is a methyl, ethyl, propyl or butyl radical.

5. A compound as claimed in any preceding claim, wherein the salt is a mineral acid salt.

6. A compound as claimed in Claim 5, wherein the mineral acid is hydrochloric acid or sulphuric acid.

7. A compound as claimed in any one of Claims 1 to 4, wherein the salt is an organic acid salt.

8. A compound as claimed in Claim 7, wherein the organic acid is α-pyrrolidone-carboxylic acid, glutamic acid, aspartic acid, lactic acid or acetic acid.

9. A compound as claimed in any preceding claim, wherein the arginine moiety is in the optically active L-form.

10. A process for producing a lower alkyl ester of mono-N-higher aliphatic acyl arginine or a salt thereof, as claimed in any one of Claims 1 to 9, which process comprises reacting arginine with a higher aliphatic acyl halide of formula RCOX where RCO is as defined in Claim 1 and X is a halogen atom, in an alkaline aqueous solution; and esterifying the resulting mono-N-higher aliphatic acyl arginine so as to introduce the lower alkyl radical R¹ as defined in Claim 1; and, if desired, converting the resulting ester to a salt of the ester.

11. A process according to Claim 10, substantially as described in the foregoing Example 10.

12. In combination, a material (other than a detergent) which is susceptible to micro-organic attack and, in association with the material, a compound as claimed in any one of Claims 1 to 9.

13. A combination as claimed in Claim 12, wherein the material is a bath preparation, a cleansing agent, a cold cream, a toilet water, a dentifrice or a cosmetic powder.

14. A combination as claimed in Claim 12, wherein the material is an alcoholic beverage.

15. A combination as claimed in Claim 12, wherein the material is formed of leather or rubber.

16. A combination as claimed in Claim 12, wherein the material is paint.

17. A combination as claimed in Claim 12, wherein the material is a foodstuff.

18. A combination as claimed in Claim 12, substantially as described in any one of the foregoing Examples 1 to 9.

19. A method of treating a material other than a detergent to reduce or prevent micro-organic attack, which method comprises applying to, or incorporating in, the material, a compound as claimed in any one of Claims 1 to 9.

20. A method according to Claim 19, substantially as hereinbefore described.

HASELTINE, LAKE & CO.,
Chartered Patent Agents,
Hazlitt House, 28, Southampton Buildings,
Chancery Lane, London, WC2A 1AT,
and 9, Park Square,
Leeds, LS1 2LH, Yorks.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.